201-14894B

Robust Summaries for

IRGANOX MD 1024

1,2-BIS(3,5-DI-TERT-BUTYL-4-HYDROXYHYDROCINNAMOYL)HYDRAZINE

CAS No. 32687-78-8

Ciba Specialty Chemicals Corporation
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CONTENTS

HEALTH ELEMENTS

SUMMARY TABLE

CAS No. 32687-78-8			
PHYSICAL/CHEMICAL ELEMENTS	**DATE**	**RESULTS**	**FULFILLS REQUIREMENT**
Melting Point		227 – 232 °C	Yes
Boiling Point	2003	741.68 °C	Yes
Vapor Pressure	2003	1.04×10^{-20} mm Hg	Yes
Partition Coefficient	2003	Log Kow > 7.79 (estimated)	Yes
Water Solubility	2003	< 1 mg/L (experimental data) 2.75×10^{-4} mg/L (calculated data)	Yes
ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS			
Photodegradation	2003	For reaction with hydroxyl radicals, predicted rate constant = 54.7×10^{-12} cm^3/molecule-sec predicted half-life = 2.3 h	Yes
Stability in Water	2003	EPIWIN model estimated extremely slow hydrolysis and t1/2 is > 1 year	Waiver
Fugacity	2003	Predicted distribution using Level III fugacity model Air 0.0271 % Water 1.23 % Soil 35.6 % Sediment 63.2 % Persistence = 5.92e+003 h	Yes
Biodegradation	1984	Not biodegradable 10 mg/L: 6% in 28 days 20 mg/L: 1% in 28 days	Yes
ECOTOXICITY ELEMENTS			
Acute Toxicity to Fish	1977	LC_{50} (96 h) > 100 mg/L	Yes
Toxicity to Aquatic Plants	1993	EC_{50} (0-72 h) > 19.8 mg/L	Yes
Acute Toxicity to Aquatic Invertebrates	1990	EC_{50} (24 h) > 15 mg/L	Yes

SUMMARY TABLE (CONTINUED)

CAS No. 32687-78-8 HEALTH ELEMENTS	DATE	RESULTS	FULFILLS REQUIREMENT
Acute Toxicity	1980	Rat: LD_{50} (Oral) > 7,000 mg/kg	Yes
	1983	Chinese Hamsters: LD_{50} (Oral) > 5,000 mg/kg	
	1972	Rat: LD_{50} (Inhalation) > 110 mg/ m^3	
Genetic Toxicity • In Vitro (Ames)	1980	Ames Test – Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 25, 75, 225, 675, and 2025 ug/0.1 ml)	Yes
• In Vivo (Nucleus Anamoly Test)	1983	No clastogenic effect in Chinese Hamster bone marrow cells	Yes
Repeated Dose Toxicity • 4- Week dietary toxicity study in rats	1983	NOEL = 10000 ppm in females	Yes
• 90-Day dietary toxicity study in rats	1984	NOEL = 400 ppm	
Developmental and Reproductive Toxicity • 90-Day Subchronic study • Teratogenicity study	1984 1983	No significant effect on reproductive organs. No teratogenic effects in the rats.	Yes

PHYSICAL/CHEMICAL ELEMENTS

1. MELTING POINT

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	Not reported
GLP:	No
Results:	227 – 232 °C
Remarks:	The melting point as reported in the MSDS from Ciba Specialty Chemicals Corp.(MSDS No. 1154, 06/13/03). The melting point was assigned a reliability code of 2g[1] (data from Handbook or collection of data).
References:	[1] Klimisch, H.J., Andreae, M and Tillman, U., A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

2. BOILING POINT

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	Since it is a solid, boiling point is estimated by the MPBPWIN Program (v.1.40) using the adapted Stein and Brown Method).[1,2]
GLP:	No
Year:	2003
Results:	741.68 °C
Remarks:	In the absence of reliable experimental data, the boiling point was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

3. VAPOR PRESSURE

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	Estimated by the MPBPWIN Program (v. 1.40) using the modified Grain method.[1, 2]
GLP:	No
Year:	2003
Results:	1.04×10^{-20} mm Hg
Remarks:	In the absence of reliable experimental data, the vapor pressure was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

4. PARTITION COEFFICIENT

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	KOWWIN Program (v. 1.66). [1, 2]
GLP:	No
Year:	2003
Results:	Log Kow = 7.79
Remarks:	The MSDS from Ciba Specialty Chemicals Corp. reported a partition coefficient of greater than 6.0, but the method of determination was not provided. In the absence of this information, the partition coefficient was calculated using an accepted method. The estimate was assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

5. WATER SOLUBILITY

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	The test conditions are adapted from the EEC directive 84/449 A.6.[1] Test solution consisted of 250 mg of Irganox MD 1024 in 500 ml Millipore water SQS HPLC quality. The saturation temperature was at 30ºC and the saturation time was 24 hours, whereas equilibrium temperature was at 20ºC. Analytical method was by photometric determination at 275 nm. Calculated values were obtained using WSKOW v1.37 model.
Temperature:	20 °C
GLP:	No
Year:	2003
Results:	< 1 mg/L (experimental data) 2.75×10^{-4} mg/L (calculated data)
Remarks:	The water solubility of the test substance is < 1mg/L at 20°C. WSKOW v1.37 [1, 2] model showed the calculated value to be 2.75×10^{-4} mg/L. In the absence of information at lower concentrations the calculated value is used. The water solubility calculated by an accepted method is assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Report on Water Solubility. 24.8.1992, Ciba-Geigy ltd., Analytical Additives AD 1.232, CH-4002 Basle, Switzerland. [2]Syracuse Research Corporation, Syracuse, NY [3]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [4]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS

6. PHOTODEGRADATION

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	Estimated by the AOP program (v. 1.87). [1,2] This model estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2003
Results:	For reaction with hydroxyl radicals, the predicted half-life of the chemical was rapid. Rate constant: 54.7×10^{-12} cm^3/molecule-sec Half-life: 2.3 h
Remarks:	In the absence of reliable experimental data, the photodegradation was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

7. STABILITY IN WATER

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	The HYDROWIN Program (v. 1.67) [1,2]
GLP:	No
Year:	2003
Results:	The HYDROWIN Program estimated that the hydrolysis rate is slow (t1/2 is > 1 year).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

8. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	Estimated by EPIWIN Level III Fugacity Model.[1,2] Fugacity model III predicts the partitioning of an organic compound in an evaluative environment, between air, soil, sediment and water using various input parameters. The model used the following inputs: Molecular weight: 552.8 Henry's Law Constant = 1.33 e-18 atm-m3/mole Vapor Pressure = 1.04e-20 mm Hg Melting Point = 325 deg C Log Kow = 7.79 Soil Koc = 2.53e+7
Year:	2003
GLP:	No
Results:	Distribution using EQC Level III Fugacity Model Air 0.0271 % Water 1.23 % Soil 35.6 % Sediment 63.2 %
Remarks:	In the absence of reliable experimental data, the fugacity was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

9. BIODEGRADATION

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was conducted under OECD Guideline 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 Evolution)," 1981. Bacteria was collected from activated sludge of a sewage treatment plant. The preparation was carried out according to the guidelines. Two liter flasks were equipped with gas inlet and magnetic stirrer. The temperature was maintained at 22 ± 2 ºC with air at approximately 50 ml/min of carbon dioxide. The only deviation from the guidelines was the amount of test solution reduced from 3 to 1.5 L. [1]
Test Type:	Aerobic
Concentration of the chemical:	Test chemical: 10 mg/ L and 20 mg/ L. Reference chemical: aniline (Merck No.1261): 20 mg/ L
Vehicle:	Water as specified in the guideline containing 0.5 ml of the nonylphenol 10EO5PO solution.
Blank:	Water as specified in the guideline.
Inoculum:	Fresh sewage treatment plant sample (per guideline)
Medium:	Sewage sludge (per guideline)
GLP:	No
Year:	1989
Results:	Test chemical: 10 mg/L: 6% degradation in 28 days 20 mg/L: 1 % degradation in 28 days Reference substance: 20 mg/L: 10 % in 28 days. Under the test conditions, no biodegradation was observed.
Conclusion:	Substance was not readily biodegradable according to OECD definition.
Remarks:	This study was assigned a reliability code of 2b [2] (guideline study with acceptable restrictions).
Reference:	[1]Report on the test for ready biodegradability of TK 10617 in the modified Sturm test, Ciba-Geigy Ltd., Basle, Switzerland, July 18, 1984. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ECOTOXICITY ELEMENTS

10. ACUTE TOXICITY TO FISH

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was conducted in accordance with the method reported by Bathe et al. (1974)[1]. Glass aquaria (12 L) were filled with reconstituted water prepared of deionised water to which salts were added. The fish were tested at 14 ± 2 ºC (trout, carp, bluegill) and at 22 ± 2 ºC (catfish, golden orfe). With the exception of experiments on trout the water was not aerated during testing. Four fish were placed in each tank with a total of 12 fish per concentration. Oxygen dissolved in the water and pH were monitored at 24 hour interval throughout the 96 hour testing period. Various concentrations of test substance were prepared by adding the test material dissolved in acetone at the appropriate amount ensuring that the total volume in each vessel remained the same. The corresponding volume of acetone was added to the vessels containing the control group.[2]
Type of test:	Static
Species:	Rainbow Trout (Salmo gairdneri), Carp (Cyprinus Carpio), Catfish (Ictalurus melas), Bluegill (Lepomis macrochirus), Golden orfe (Leuciscus idus melanotus)
Average Length:	101 mm (Rainbow Trout) 71 mm (Carp) 56 mm (Catfish) 56 mm (Bluegill) 76 mm (Golden Orfe)
Average Weight:	11.1 g (Rainbow Trout) 5.8 g (Carp) 2.6 g (Catfish) 3.6 g (Bluegill) 3.1 g (Golden Orfe)
Exposure period:	96 h

Exposure Concentrations:

Species	Test Concentrations
rainbow trout	37, 65, 100 mg/L
carp	49, 100 mg/L
cat fish	37, 100 mg/L
bluegill	49, 100 mg/L
golden orfe	37, 100 mg/L

Analytical Monitoring:	No
GLP:	No
Year:	1977
Results:	LC_{50} (96 h) > 100 mg/L (nominal) for Rainbow Trout, Carp, Catfish, Bluegill, and Golden Orfe. Mortalities in Blank and Vehicle: 0% Mortalities in Treatment Group: 0%
Remarks:	This study was assigned a reliability code of 2c[3] (comparable to guideline study with acceptable restrictions).
Reference:	[1] R. Bathe, k. Sachsse, L. Ullmann, W.D. Hormann, F. Zak and R. Hess: The evaluation of Fish toxicity in the laboratory. In: Proceedings of the Europ. Soc. Of Toxicology. Vol XVI, pp. 113-124, Carlsbad, June 1974. [2]Acute Toxicity to Rainbow Trout, Carp, Catfish, Bluegill, and Golden Orfe of TK 10617. Project No. Siss 5846, Ciba-Geigy Ltd., Basel, Switzerland, January 14, 1977. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

11. TOXICITY TO AQUATIC PLANTS

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was conducted under test guideline: 87/302/EEC page 89-94, Algal growth inhibition test. The static *scenedesmus subspicatus* toxicity screen was conducted in 100-mL Erlenmeyer flasks containing 50 mL of algae nutrient media or test solution. Each test concentration was tested in triplicate and the blank control in 6. The temperature was maintained at 23 ± 0.5 º C, pH was measured at 0 and 72 h exposure and with continuous illumination, cold white fluorescent light, and 125 µE/m3 sec (approx. 8000 lux). Cell densities were measured at 24, 48, 72 hours.[1]
Species:	Green Algae (*Scenedesmus subspicatus*)
Test Procedure:	Static
Age of Culture at Study Initiation:	3 days old
Test concentrations:	1.23, 3.7, 11, 33 and 100 mg/L (nominal)
Vehicle:	4.0 mg Tween 80/L (polyoxyethylene-sorbitan-mono-oleate)
Blank:	Water
Exposure period:	72 h
Analytical Monitoring:	No
GLP:	No
Year:	1993
Results:	EC_{50} (0-72 h) = 19.8 mg/L 95% confidential limit: 13.6 –25.1 mg/L NOEbC (0-72 h) (1% level): <1.23 mg/L Values are based on nominal concentrations.
Remarks:	This study is assigned a reliability code of 2b[2] (guideline study with acceptable restrictions).
Reference:	[1]Report on the growth inhibition test of Irganox MD 1024 to green algae (Scenedesmus subspicatus); Dr. R. Grade, Dr. A.von Schulthess; Ciba-Geigy, Ltd., Basel, Switzerland; January 20, 1993. [2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

12. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was conducted under the Guideline: Official Journal of the European Communities L 251, vol. 27, C-02, 19/09/1984. Cultures of daphnia are maintained in glass vessels containing approx. 2.5 L of reconstituted water. The temperature was maintained at 20 ± 1° C (water is renewed partially 3 times weekly), lighting was fluorescent light, 16 hours daily approx. 1500 lux. 24 hours before the beginning of the exposure reproductive daphnia were separated from the young by sieving all individuals through a 800 µm sieve. The young were retained for the test. The calculated amounts of the stock solution required to produce the test concentrations were added to the water and were homogeneously distributed. The daphnia were then transferred into the beakers. The oxygen content, pH, and temperature were measured at 0, and 24 hours.[1]
Species:	*Daphnia magna Straus 1820*
Type of test:	Static
Test Concentration:	1.0, 1.8, 3.2, 5.8, 10, 18, 32, 58, 100 mg/L (nominal)
Control:	Blank: Water
Number of Daphnia:	20/ concentration and control 4 replicates of 5 daphnia each
Exposure period:	24 hours
Analytical monitoring:	No
GLP:	No
Year:	1990
Results:	EC_{50} (24 h): 15 mg/L EC_0 = 1.8 mg/L Values are based on nominal concentrations. Immobilization in blank = 0%
Remarks:	The study is assigned a reliability code of 2b[2] (guideline study with acceptable restrictions).
Reference:	[1]Report on the acute toxicity test of Irganox MD 1024 to Daphnia (Daphnia magna Straus 1820), test No.: 904214, Ciba-Geigy Ltd., Basle, Switzerland, November 09, 1990.

[2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

HEALTH ELEMENTS

13. ACUTE TOXICITY

A. Oral

i. In Rat

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	The test substance was suspended in polyethyleneglycol (PEG 400). Before treatment the suspension was homogeneously dispersed with an ultra-turrax and during treatment it was kept stable with a magnetic stirrer. Healthy random bred rats of the Tif: RAIf (SPF) strain raised on the premises were used. They were kept at room temperature of 22 ± 1° C, at a relative humidity of 55 ± 5% and on a 10 hours light cycle day. They received ad libitum rat food and water. During the treatment and observation period the animals were housed in groups of 5 in Macrolon cages. Physical condition and rate of deaths were monitored throughout the whole observation period. [1]
Species/strain:	Random bred rats of the Tif: RAIf (SPF) strain
Age at Initiation:	7 to 8 weeks old
No. Animals/Group:	5 / dose level
Dose:	4000, 5000, 6000, 7000 mg / kg
Vehicle:	Polyethylene glycol (PEG 400)
GLP:	No
Year:	1980
Results:	LD_{50} > 7,000 mg /kg The animals in all dosage groups showed symptoms of sedation, dyspnea, curved position and ruffled fur. The animals recovered within 8 days. They were submitted to a necropsy at the end of the observation period. No substance related gross organ changes were seen. No mortalities were observed.

Remarks:	The study was assigned a reliability code of 2[2] (valid with restriction).
Reference:	[1]Acute Oral LD_{50} in the Rat, Ciba-Geigy Ltd., March 19, 1980.
	[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii. In Chinese Hamster

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	The test was conducted under OECD guidelines No. 401. Healthy random bred Chinese hamsters were used. The animals were kept under conventional laboratory conditions. They were kept at room temperature of 22 ± 3 °C, at a relative humidity of 55 ± 5% and on a 12 hours light cycle day with approximately 15 air changes/h. They received ad libitum hamster food, NAFAG No.923/24, NAFAG AG, Gossau, SG (Switzerland) and water. During the treatment and observation period the animals were housed individually in Macrolon cages type 2. Prior to dosing, the animals were fasted overnight. Physical condition and rate of deaths were monitored throughout the whole observation period. [1]
Species/strain:	Random bred Chinese hamsters
Age at Initiation:	9 to 11 weeks old
Initial Body weight range:	21-35 g
No. Animals/Group:	5 males and 5 females/ dose level
Dose:	5000 mg / kg
Administration:	oral, by gastric intubation (gavage)
GLP:	No
Year:	1983
Results:	LD_{50} > 5000 mg /kg The animals in the dose group showed symptoms of sedation, dyspnea, curved position and ruffled fur. Spontaneously dying animals and the survivors were submitted to a necropsy at the end of the observation period. No substance related gross organ changes were seen. 20 % of deaths were recorded in higher dose group.
Remarks:	The study was assigned a reliability code of 2b[2] (Guideline study with acceptance restrictions).
Reference:	[1]Acute Oral LD_{50} in the Chinese Hamster. GU Project No. 821615, Ciba-Geigy Ltd.,March 15, 1983. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5,1997.

B. Inhalation

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	Young adult albino rats were housed individually in stock cages and permitted a standard laboratory diet plus water ad libitum, except during inhalation exposure. Test animals were exposed in a specially constructed Plexglas inhalation chamber having a capacity of 700 liters. The test atmosphere was generated by heating 7.0 g of test material in an aluminium pan on a hot plate, placed in an empty 700-liters chamber. The resulting fumes and vapors were then drawn into the 700-liter chamber containing the test animals. Air flow rate through the system was 150 L/min. The temperature of the test atmosphere was 32^{o} C and the pressure was 29.71 inches Hg. The average concentration, obtained by repeated air sampling, was 110mg/m^{3} air. Following 4 hour exposure, the test animals were observed for a period of 14-days.[1]
Type:	Acute inhalation - mist
Species/strain:	Young adult albino rats (Sprague-Dawley strain)
Initial Body Weight Range:	154 g
Total number of animals:	5 males and 5 females
Dose level:	110 mg/m^{3}
Exposure time:	4 hours
GLP:	No
Year:	1972
Results:	No deaths or untoward behavioral reactions were noted among any test animals. No adverse effects on body weight were noted. The average two-week body weight gain was 73 g which is within the normal range. Necropsy of all animals sacrificed at the end of the 14-day observation period revealed moderate lung hyperemia in five rats. No gross pathologic alterations were observed in any of the other tissues and organs examined.
Remarks:	The study was assigned a reliability code of 2c[2] (comparable to guideline study with acceptable restrictions).

Reference:

[1]Acute inhalation toxicity study with in Albino Rats; June 12, 1972; IBT No. N1596, Industrial BIO-TEST Laboratories, Inc., Illinois.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

14. GENETIC TOXICITY IN VITRO

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was conducted using the methods described by Ames *et al* (1973, 1975) [2,3,4]. The material was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537 and TA 1538). The investigations were performed with and without microsomal activation. The tests were performed with and without microsomal activation at the following concentrations: 25, 75, 225, 675, and 2025 ug/0.1 ml. The test substance was dissolved in DMSO. Positive controls were carried out simultaneously. In the experiments without and with the addition of microsomal activation mixture three petri dishes were prepared per strain and per group. The plates were incubated for about 48 hours at 37 ^{o} C in darkness. When the colonies had been counted, the arithmetic mean was calculated.[1]
Type:	Bacterial mutagenicity
System of testing:	Salmonella typhimurium TA 98, 100, 1535, 1537 and 1538
Positive Control:	daunomycin-HCl - for strain TA98, 4-nitroquinoline-N-oxide - for strain TA100 N-methyl-N'-nitro-N-nitosoguanidine - for strain TA1535 9 (5)aminoacridine hydrochloride monohydrate – for strain TA1537 2-nitrofluorene – for strain TA1538
Solvent Control:	DMSO
Test Concentrations:	25, 75, 225, 675, and 2025 ug/0.1 mL
GLP:	No
Year:	1980
Results:	Comparison of number of histidine-prototrophic mutants in the control and treated group did not reveal any marked differences. The slight increase in the number of back-mutant colonies in the experiment on strain TA 1535 with microsomal activation is attributed to variations in the rate of spontaneously occurring back-mutants. At the concentrations of 675 and 2025 ug/0.1 mL the substance precipitated in soft agar. Number of colonies of histidine-prototrophic back-mutants with and without microsomal activation are summarized in the table below.

Number of colonies of histidine-prototrophic back mutants (arithmetic mean)
(without microsomal activation)

	Concentration (ug/0.1 ml)	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Test substance	Control	11	136	8	4	12
	25	20	129	10	3	8
	75	18	158	10	7	11
	225	13	145	10	6	6
	675	16	156	7	2	12
	2025	17	159	7	6	9
Positive controls						
Daunorubicin-HCl	Control	19				
	5	134				
	10	293				
4-Nitroquinoline-N-oxide	Control		156			
	0.125		650			
	0.25		>1000			
N-methyl-N'-nitro-N-nitrosoguanidine	Control			8		
	3			349		
	5			~1470		
9(5) Aminoacridine hydrochloride	Control				6	
	50				85	
	100				520	
2-Nitrofluorene	Control					11
	5					660
	10					823

Number of colonies of histidine-prototrophic back mutants (arithmetic mean)
(with microsomal activation)

	Concentration (ug/0.1 ml)	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Test substance	Control	26	141	4	3	15
	25	24	136	7	4	15
	75	21	141	12	5	19
	225	26	128	7	5	16
	675	24	112	7	3	16
	2025	21	130	8	5	17

Positive control						
Cyclosphosphamide	Control			13		
	250			214		

Conclusion: No mutagenic effects were observed.

Remarks: This study was assigned reliability code of 2e (met generally accepted scientific standards, was well documented, and was acceptable for assessment).[5]

References:

[1]Salmonella/Mammalian-Microsome Mutagenicity Test. Ciba-Geigy Ltd, Basel, Switzerland, September 15, 1980.

[2]Ames, B.N., Lee, F.D., and Durston, W.E., "An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. USA, 70, 782-786, 1973.

[3]Ames, B.N., Durston, W.E., Yamasaki, E., and Lee, F.D., "Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection," Proc. Natl. Acad. Sci. USA, 70, 2281-2285, 1973.

[4]Ames, B.N., McCann, J., and Yamasaki, E., "Methods for detecting carcinogens and mutagens with the Salmonella / mammalian-microsome mutagenicity test, Mutat. Res., 31, 347-364, 1975.

[5]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

15. GENETIC TOXICITY IN VIVO

Nucleus Anomaly Test in Chinese Hamster Bone Marrow

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	The experiment was done to evaluate any mutagenic effects on somatic interphase cells in vivo. Chinese hamsters of both sex were used. The animals were kept in an air-conditioned room at a temperature of 20-23 ^{o} C, and a relative humidity of 49-60%. The room was illuminated for 12 hours daily. Water is tap water ad libitum and standard diet NAFAG No. 924. The test material was administered orally to groups of 6 female and 6 male animals each. Treatment consisted of one daily application on 2 consecutive days. 24 hours after the second application the animals were sacrificed by dislocation of the cervical vertebrae. Bone marrow was harvested from the shafts of both femurs.[1]
Species/strain:	Chinese Hamster (Circetulus griseus) random outbred strain
Age at Initiation:	Males: 6-10 weeks Females: 4-9 weeks
Initial Body Weight Range:	males: 20 –27 g Females: 22-30 g
Total number of animals:	6 males and 6 females / group
Rout of administration:	Oral by stomach tube
Dose level:	0, 1250, 2500, and 5000 mg/kg
Control:	Positive control: Cyclophosphamide Negative control: Arachid oil
GLP:	No
Year:	1983
Results:	The bone marrow smears from animals treated with the selected doses of test material showed no significant difference from the control. A significant positive response was detected in the bone marrow smears of rats receiving cyclophosphamide. The effect of test substance and cyclophosphamide on Bone marrow cells of Chinese Hamster are shown in the table below.

Table

The effect of test substance and cyclophosphamide on Bone marrow cells of Chinese Hamster

Percent of cells with anomalies of nuclei

Group	No. of animals	Sex of animals	Single Jolly bodies	Fragments of nuclei in erythrocytes	Micronuclei in erythroblasts	Micronuclei in leucopoietic cells	Polyploid cells	Total
Control (CMC 0.5%)	1	♀	0.2					0.2
	2	♀	0.2					0.2
	3	♀	0.1					0.1
	4	♂						0.0
	5	♂	0.2					0.2
	6	♂	0.3					0.3
Cyclophosphamide (128 mg/kg)	1	♀	7.7	0.3	1.0	0.3		9.3
	2	♀	6.5	0.2	1.5	0.8	0.1	9.1
	3	♀	7.9	0.7	0.3	0.1		9.0
	4	♂	4.9	0.2	0.7	0.1	0.1	6.0
	5	♂	3.9		0.4	0.4		4.7
	6	♂	4.6	0.2	0.3	0.1		5.2
TK 10617 1250 mg/kg	1	♀						0.0
	2	♀	0.1					0.1
	3	♀	0.1					0.1
	4	♂				0.1		0.1
	5	♂						0.0
	6	♂	0.1					0.1
TK 10617 2500 mg/kg	1	♀	0.1					0.1
	2	♀						0.0
	3	♀						0.0
	4	♂	0.1					0.1
	5	♂						0.0
	6	♂						0.0
TK 10617 5000 mg/kg	1	♀	0.1					0.1
	2	♀						0.0
	3	♀	0.2					0.2
	4	♂						0.0
	5	♂	0.1					0.1
	6	♂	0.1					0.1

Conclusion:	No evidence of mutagenic effects were seen in Chinese hamsters treated with test substance.
Remarks:	This study was assigned reliability code of $2c^2$ (comparable to guideline study with acceptable restriction).
References:	[1]Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster.October 4, 1983, Ciba-Geigy Ltd. Basle, Switzerland. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

16. REPEATED DOSE TOXICITY

Subchronic Toxicity:

i) 28-Day Dietary Toxicity study in Rats:

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	In this study a total of 80 RAIF (SPF) rats, 10 males and 10 females/dose group were used. The test article was administered in the diet for 28 days at a concentration of 0, 1000, 3000 and 10,000 ppm. The experiment was carried out under specified pathogen free (SPF) standard laboratory conditions. The rats were housed in groups of 5 in Macrolon cages type 4 with standardized granulated soft wood bedding. The animal room was air conditioned at a temperature of 22 ± 2 ^{o}C, and a relative humidity of 55 ± 10% and on a 12 hours light cycle day with approximately 15 air changes/h. Pelleted certified standard diet Nafag No. 890 Tox was fed ad libitum. Abnormal reactions and deaths were recorded daily. Body weight and food consumption were measured weekly. This study was conducted as a dose range finding and preliminary toxicity study for the 90-day rat study.[1]
Species/strain:	Tif: RAIF (SPF) rats
Initial age:	~ 4 weeks old
No. of animals:	10 males and 10 females/ group (total 80)
Route of administration:	Dietary
Exposure period:	28 days
Dose:	0, 1000, 3000, and 10,000 ppm in food
GLP:	No
Year:	1983
Results:	No mortality and no clinical symptoms and no signs of local and/ or systemic toxicity were observed. There were no abnormalities in body weight gain, and food consumption rate when compared to the control group. Macroscopic examination at autopsy revealed no evidence of a reaction to the treatment.

Conclusion:	The no observable effect level was found to be above 10,000 ppm, corresponding to a mean daily intake of 1013 mg/kg body weight for males and 936 mg/kg bw for females
Remarks:	This study was assigned a reliability code of 2c[2] (comparable to guideline study with acceptable restrictions)
Reference:	[1] 28-Day Palatability study in Rats. GU Project No.821055, Ciba -Geigy Limited, Basel, Switzerland, January 05, 1983.
	[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997

ii) 3-Month Toxicity study in Rats:

Test substance:	1,2-bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was carried out under OECD guidelines for testing of chemicals, sudchronic oral toxicity-Rodent: 90-day study, No. 408, adopted May 12, 1981 and in accordance with the OECD Principles of Good Laboratory Practice (GLP), adopted May 12, 1981 by the OECD council and was subjected to periodic quality assurance evaluation. The experiment was carried out under specified pathogen free (SPF) standard laboratory conditions. The rats were housed in groups of 5 in Macrolon cages type 4 with standardized granulated soft wood bedding. The animal room was air conditioned at a temperature of 22 ± 2 ^{o}C, and a relative humidity of 55 ± 10% and on a 12 hours light cycle day with approximately 16-20 air changes/h. Pelleted certified standard diet Nafag No. 890 Tox, and tap water were allowed ad libitum. The compound was administered at a dose level of 0, 400, 2000, and 10,000 ppm by mixing in the food. Mortalities and clinical symptoms were recorded daily; food consumption and body weights were recorded weekly. Water consumption monthly. At the end of the test period all animals were subjected to detailed autopsy. Liver, adrenals, brain, thymus, heart, kidneys and gonads were weighed. An histopathological examination was carried out on the following organs: skin, mammary area, spleen, mesenteric lymph node, axillary lymph node, sternum with bone marrow, femur with joint, skeletal muscle, trachea, lung, heart, sorta, submandibular salivary gland, liver, pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, prostate, seminal vesicle, testis, epididymis, uterus, ovary, pituitary gland, adrenal gland, thyroid with parathyroid gland, thymus, peripheral nerve, brain, spinal cord, eye with optic nerve, orbital gland, extraorbital lacrimal gland, organs and tissues showing macroscopic changes.[1]
Species/strain:	Tif:RAIf (SPF) rats, F3-hybrid of RII 1/Tif x RII 2/Tif
Initial age:	6 weeks
Initial weight:	190-195 g (males) 173-175 g (females)
No. of animals:	20 males and 20 females (total 160)
Route of administration:	Dietary
Exposure period:	3 months
Dose:	0, 400, 2000, and 10,000 ppm

	Males: 25, 123 and 624 mg/kg Females: 27, 127 and 667 mg/kg
GLP:	Yes
Year:	1984
Results	The mean body weight gain of all treated male and female groups was similar to that of the respective control groups, except a marginally lower weight increase in treated male group 4 (10,000 ppm), which however was not significant and within a range of 10% of the control value. This correlates with testes weight loss, where a slight decrease of testes weight was noted in treated male group 4 (1000 ppm). The mean food consumption, mean water consumption of all treated groups was similar to that of control group. No mortalities, no clinical symptoms and no signs of local and/or systemic toxicity were observed. An ophthalmic and hearing examination showed no abnormalities in any group. Haematological investigations at week 14 showed a minimal increase in numbers of thrombocytes in females of group 4 (10,000 ppm). The total protein concentration and the albumin concentration were noted to be marginally lower in the females of group 4 (10,000 ppm). The globulin concentration of this group was slightly increased. The toxicological significance of these findings is doubtful. Mean organ weights and ratios are presented in the table below. A slight, but significant decrease of the liver weight was observed in treated male rats of groups 3 and 4 (2000 and 10000 ppm). A slight decrease of testes weight was noted in treated male group 4 (10000 ppm). This organ weight loss may have resulted from the general stress that reduced male body weights in this high dose group. There were no histopathological effects noted for the testes. Statistically significant differences in organ weights, as indicated by asterisks in the following tables, between treated and control groups were noted. Since no systemic pattern emerged; these differences were attributed to spontaneous variation rather than to the treatment.

Table

Mean Organ Weights (g) and Ratios (as a percentage of body- and brain weight)

Male Rats (Test Week 14)

ORGANS	DOSE IN PPM							
	0.0		400.0		2000.0		10000.0	
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN
Body	20	457.209	20	460.444	19	449.525	20	435.369
Brain	20	2.402	20	2.417	19	2.446	20	2.441
Brain / Body	20	0.528	20	0.529	19	0.548	20	0.563
Heart	20	1.367	20	1.285	19	1.279	20	1.308
Heart / Body	20	0.300	20		19	0.285	20	0.301
Hearts / Brain	20	56.994	0.280*		19		20	53.565
			20		52.375*			
			53.503*					
Liver	20	14.975	20	16.113	19	13.926	20	12.363*
Liver / Body	20	3.274	20		19	3.100	20	2.845*
Liver / Brain	20	624.655	3.509*		19		20	506.811*
			20	670.299	570.909*			
Kidneys	20	2.785	20	2.788	19	2.795	20	4.048*
Kidneys / Body	20	0.610	20	0.610	19	0.622	20	0.850
Kidneys / Brain	20	116.120	20	115.970	19		20	112.411
					114.384*			
Adrenals	20	0.068	20	0.070	19	0.066	20	0.068
Adrenals / Body	20	0.0148	20	0.0152	19	0.0147	20	0.0157
Adrenals / Brain	20	2.834	20	2.909	19	2.704	20	2.783
Thymus	20	0.410	20	0.426	19	0.438	20	0.456
Thymus / Body	20	0.090	20	0.093	19	0.098	20	0.105
Thymus / Brain	20	17.102	20	17.831	19	17.958	20	18.720
Gonades	20	3.821	20	3.530	19	3.616	20	3.425*
Gonades / Body	20	0.840	20	0.779	19	0.809	20	0.792
Gonades / Brain	20	159.183	20	147.206	19		20	140.611*
					148.293*			

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE BETWEEN CONTROL AND TREATMENTGROUP (< 0.050)

Mean Organ Weights (g) and Ratios (as a percentage of body- and brain weight)

Female Rats (Test Week 14)

ORGANS	DOSE IN PPM							
	0.0		400.0		2000.0		10000.0	
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN
	TREND							
Body	20	302.284	20	312.354	20	315.589	20	298.740
Brain	20	2.328	20	2.325	20	2.356	20	2.317
Brain / Body	20	0.774	20	0.749	20	0.757	20	0.780
Heart	20	0.972	20	0.974	20	0.970	20	0.916
Heart / Body	20	0.322	20	0.313*	20	0.310*	20	0.308
Hearts / Brain	20	41.768	20	41.992	20	41.293	20	39.656
Liver	20	10.735	20	10.599	20	10.326	20	10.307
Liver / Body	20	3.556	20	3.399	20	3.289*	20	3.452
Liver / Brain	20	461.254	20	455.404	20	438.469	20	446.063
Kidneys	20	2.063	20	1.982	20	2.040	20	2.078
Kidneys / Body	20	0.687	20	0.636*	20	0.651	20	0.698
Kidneys / Brain	20	88.636	20	85.324	20	86.525	20	89.833
Adrenals	20	0.093	20	0.087	20	0.094	20	0.088
Adrenals / Body	20	0.0308	20	0.0282	20	0.0300	20	0.0298
Adrenals / Brain	20	3.987	20	3.761	20	4.003	20	3.823
Thymus	20	0.303	20	0.315	20	0.312	20	0.286
Thymus / Body	20	0.101	20	0.101	20	0.099	20	0.096
Thymus / Brain	20	13.080	20	13.593	20	13.330	20	12.388
Gonades	20	0.174	20	0.190	20	0.175	20	0.177
Gonades / Body	20	0.058	20	0.062	20	0.056	20	0.060
Gonades / Brain	20	7.495	20	8.196	20	7.419	20	7.662

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE BETWEEN CONTROL AND TREATMENT GROUP (< 0.050)

Histopathology: There were no macroscopic or microscopic changes observed except for an increase in incidence of non-specific and minimal inflammatory cell infiltration in the liver in male animals of treated groups 3 and 4 (2000 and 10000 ppm) which was considered to be due to the administration of the test substance.

The NOEL is 400 ppm, corresponding to a mean daily intake of 25 mg/kg body weight for males and 27 mg/kg bw for females.

Remarks: This study was assigned a reliability code of 2b[2] (Guideline study with acceptable restrictions).

Reference: [1]3-Month Toxicity Study in Rats, Final Report, January 20, 1984. Ciba Geigy Limited, Basel, Switzerland

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

17. DEVELOPMENTAL / REPRODUCTIVE TOXICITY

A. DEVELOPMENTAL TOXICITY IN RATS

Test substance:	1,2-bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamoyl)hydrazine CAS No. 32687-78-8
Method:	This study was carried out under OECD guidelines for testing of chemicals, No. 414 (Teratogenicity). The compound was administered by oral gavage on Days 6 through 15 of gestation. Throughout the experiment the successfully mated females were kept in groups of 4 in Macrolon cages equipped with a wire mesh top and water bottles, saw dust (granular form) serving as bedding material. The cages were placed in movable racks kept in an air-conditioned room at a temperature of 21± 2 ^{o} C and a humidity of 55 ± 10 %. The room was illuminated for 12 hours daily. A certified standard cube diet and tap water was available at all times throughout the experiment. During the treatment, general condition, weight gain and symptoms were checked daily. Food consumption was noted on days 6, 11, 16 and 21 of pregnancy. Dams were killed, and fetuses removed by cesarean section on day 21 of gestation[1]. The examination of dams and progeny was carried out in accordance with the technique described by Wilson, 1965 and Dawson. [2,3,4] Following assessment of the dams' organs, especially of the ovaries and uterus (mucosa and contents, including amniotic fluid and placenta as well as abortions and resorption sites) the foetuses were removed, sexed, and subjected to careful eternal inspection. They were then weighed individually and submitted to examination of viscera according to slicing technique of Wilson and skeletal assessment according to the technique of Dawson.
Species/strain:	Sprague-Dawley derived (Tif : RAlf(SPF)) rats
Sex/ age:	Female, 2 months of age.
Body weight:	180-200 g
No. of animals:	24 females/dose group
Route of administration:	Orally by intubation
Exposure period:	Days 6 through 15 of pregnancy
Doses:	0, 500, 1500, 3000 mg/kg of body weight

Vehicle: a mixture of one part of distilled water and one part of Polyethylene glycol 400.

GLP: No

Year: 1983

Results: Throughout the period of treatment food consumption and body weight gain were comparable to the control groups.

The implantation rates, embryo-lethality and/or feto-lethality (resorption) were comparable for all groups. The number of embryonic and/or fetal deaths (resorptions) were not increased at either dose. The male to female ratios of the fetuses were also comparable for all groups.

In comparison with the vehicle control, the average body-weight of the live fetuses was not diminished at either dose (Student's t test, one-tailed, observed $p < 0.01$). Details are shown in Table 1.

Table 1
Summarized Reproduction data

Dose Group (mg/kg/d)	**0**	**500**	**1500**	**3000**
Number of spontaneous deaths	0/24	0/24	0/24	0/24
Body-weight gain (%)	+14.6	+13.3	+16.7	+13.9
No. of females with deciduomata *	0/24	0/24	0/24	0/24
No. of females with implantations **	22/24	22/24	23/24	20/24
Implantations/female (mean, S.D) **	15.9 ± 2.5	16.3 ± 1.5	15.4 ± 1.9	16.1 ± 1.8
No. of females with total abortion	0	0	0	0
No. of females with partial abortion	0	0	0	0
Aborted embryos in % of implantations	-	-	-	-
Embryonal deaths (resorption) ***	6.3	4.2	6.5	8.4
Fetal deaths (resorption) ***	0	0.3	0	0
Dead fetuses ***	0	0	0	0
No. of live fetuses (males – females)	163-165	158-185	164-167	150-144
Percent males of live fetuses	49.7	46.1	49.5	51.0
Average weight of live fetuses in grams	5.4 ± 0.4	5.3 ± 0.5	5.4 ± 0.5	5.4 ± 0.5

* Haemorrhagic alterations of implantations sites
** Other than deciduomata
*** In % of total number of implantations (minus abortions)

Fusion of placentas, the implantation sites consisting of a embryonic resorption and a poorly developed male fetus, respectively was noted for one female of 500 mg/kg group.

The gross inspection of the live fetuses revealed one fetus with omphalocele in the low-dose group (see table 4).

In carrying out the slicing technique for visceral examination, dilation of the renal pelvis (unilateral) was found in one fetus of the vehicle control, data are summarized in table 4 and in table 2.

Table 2 (Visceral Assessment)

Dose Group (mg/kg)	No. of live fetuses examined			No. of fetuses affected
	Male	Female	Total	
0	65	44	109	1 male*
500	55	59	114	0
1500	69	40	109	0
3000	57	42	99	0
Historical (cumulative) control	4662	4888		11 males, 7 females **

* for findings refer to Table 4
** for findings refer to Table 5

The skeletal assessment revealed instances of irregular sternebral ossification in the dose groups and in the vehicle control. Abnormal wide suture was found in one fetus of the high dose group. Data are summarized in tables 3 and 4.

Concerning the status of skeletal maturation of the fetuses shortly before term, a slight delay in ossification of the phalangeal nuclei of the hind-limbs was recorded for the high dose group in comparison with the control group; this finding however, is not assumed to be of experimental significance.

Table 3 (Skeletal Assessment)

Dose Group (mg/kg)	Number of Skeletons examined	Phalangeal Nuclei [a]		Calcaneus [b]	5th Sternebrae [c]	Vertebrae [d]	Vertebrae [e]	Skeletal anomalies [f,g,i]
		Fore-limb	Hind-Limb					
0	219	32 (14.6)	99(45.2)	196 (89.5)	33 (15.1)	3(1.4)	0 (0.0)	2 (0.9) [f]
500	229	48 (21.0)	115(50.2)	199 (86.9)	33 (14.4)	4(1.7)	1 (0.4)	5 (2.2) [f]
1500	222	23 (10.4)	112(50.5)	202 (91.0)	45 (20.3)	7 (3.2)	3 (1.4) [h]	3 (1.4) [f]
3000	195	18 (9.2)	108(55.4)	183 (93.8)	19 (9.7)	7 (3.6)	1 (0.5)	3 (1.5) [i]
99% Confidence limits of the vehicle Control	-	(9.1-1.9)	(37-54.4)	(85.0-93.8)	(9.5-22.4)	(0.2-5.0)	(0.0-2.4)	-
Cumulative control	6180	(0.0-10.7)	(4.5-65.6)	(6.8-90.3)	(3.0-88.3)	(0.0-6.9)	14 (0.23)	32 (0.52) [g]

* Figures in parentheses refer to % skeletal anomalies

a) Ossification still absent in proximal phalahges, digit 5
b) Ossification still absent
c) Still incompletely ossified
d) Some thoracic vertebral centers still dumbbell shaped
e) Some thoracic vertebral centers bipartite
f) For details refer Table 3
g) For details refer Table 4
h) Four litters effected
i) One fetus effected

Table 4
List of Anomalies and/or malformations recorded

Dose Group (mg/kg)/ hour	Type of malformation/ anomaly	Litter affected	Live fetuses affected	%
0	Renipelvic dilatation (unilat)	(b) 1/22	1/109	(0.9)
	Sternebrae 1+2 bipartite	(c) 1/22	1/219	(0.5)
	5th sternebra bipartite	(c) 1/22	1/219	(0.5)
500	Omphalocele	(a) 1/22	1(d)/343	(0.3)
	Sternebrae 1+2 irregularly ossified	(c) 1/22	1/229	(0.4)
	Sternebrae 1+2 fused and irregularly shaped	(c) 1/22	1/229	(0.4)
	Sternebrae 2+5 bipartite	(c) 1/22	1/229	(0.4)
	4th sternebra bipartite	(c) 1/22	1(d)/229	(0.4)
	Sternebrae 3-6 bipartite	(c) 1/22	1/229	(0.4)
1500	1st Sternebrae irregularly ossified	(c) 1/23	1/222	(0.5)
	Sternebrae 1+2 fused and irregularly shaped	c) 1/23	1/222	(0.5)
	2nd sternebra bipartite	c) 1/23	1/222	(0.5)
3000	Abnormal “wide suture”	(c) 1/20	1(e)/195	(0.5)
	1st Sternebrae irregularly ossified	(c) 1/20	1(e)/195	(0.5)
	3rd sternebra bipartite	(c) 1/20	1(e)/195	(0.5)

(a) Gross inspection (b) Visceral examination (c) Skeletal assessment
(d) One litter (e) One foetus.

Table 5

List of Anomalies and/or malformations recorded for the historical (cumulative) control

Type of malformation/ anomaly	No. of live fetuses affected	%
General oedema*	2/9550 (1/4662m, 1/4888f)	0.021
Omphalocele*	4/9550 (2, 1[4] /4662m, 1/4888f)	0.042
Agnathia inferior (mandibular aplasia) *	5/9550 (1, 1[4] /4662m, 1/4888f)	0.052
Hypognathia inferior (mandibular hypoplasia)*	1, 1[1]/9550 (1 /4662m, 1[7], 1/4888f)	0.031
Cleft palate *	1[2]/9550 (1/4662m)	0.01
Hydrocephaly **	1[1]/3559 (1/1694m)	0.03
Cranioschisis *	1[2]/9550 (1/4662m)	0.01
Meningocele **	1/3559 (1/1694m)	0.03
Rhinencephaly *	1[2]/9550 (1/4662m)	0.01
Anophthalmia (bilateral) *	1[1]/9550 (1/4662m)	0.01
Exophthalmia (bilateral) *	1[6]/9550 (1/4662m)	0.01
Microphthalmia **	1[1]/3559 (1/1694m)	0.03
Eye showing double-lens (unilateral) **	1/3559 (1/1694m)	0.03
Open eyes (unilateral)*	1[6], 1[2] /9550 (2/4662m)	0.021
Supernumerary hind-limb *	1[4] /9550 (1/4662m)	0.01
Dystopia cordis and hypoplasia of lungs **	1/3559 (1/1694m)	0.03
Anasarca (slight oedema-like changes of subcutaneous tissue) **	3, 1[3]/3559 (2/1694m, 2/1665f)	0.119
Yellowish discoloration of the liver **	5[5]/3559 (3/1694m, 2/1665f)	0.149
Small cyst of the liver **	1/3559 (1/1665f)	0.03
Caudal displacement of left kidney **	2[5]/3559 (1/1694m, 2/1665f)	0.060
Renipelvic dilation ** (left)	1[5]/3559 (1/1694m)	0.03
Irregularly shaped 1st sternebra ***	4/6180	0.065
Irregularly shaped 3rd sternebra ***	1/6180	0.016
Irregularly shaped 4th sternebra ***	1/6180	0.016
Irregularly shaped 5th sternebra ***	2/6180	0.032
Irregularly shaped 6th sternebra ***	1/6180	0.016
Supernumerary ossification center between sternebrae 5+6 ***	2/6180	0.032
Irregular ossification of sternebrae 1-3 ***	1/6180	0.016
Irregular ossification of sternum ***	1[7], 5/6180	0.097
Marginal sysnostosis of sternebrae 1+2 ***	1/6180	0.016
sysnostosis of sternebrae 2+3 ***	2/6180	0.032
sysnostosis of sternebrae 4+5 ***	1/6180	0.016
Partial fusion of irregularly shaped sternebrae 4+5 ***	1/6180	0.016
Unilateral costal fusion + synostosis of vertebral arches ***	1/6180	0.016
Unilateral synostosis of 2 lumbar vertebral arches ***	1/6180	0.016
Only one half of a thoracic vertebral center ossified ***	5/6180	0.081
Wavy ribs ***	1/6180	0.016
Additional rib (14th, bilateral) ***	1/6180	0.016

* gross inspection
** visceral examination
*** skeletal assessment
m males
f females
[1] one fetus
[2] one fetus
[3] one fetus
[4] one fetus
[5] from one litter
[6] one fetus
[7] one fetus

Conclusion:	Test compound did not exhibit either a teratogenic potential or an increased embryo-lethality rate in the albino rats under the experimental conditions.
Remarks:	This study was assigned a reliability code of 2b[5] (guideline study with acceptable restrictions).
References:	[1]Teratology Study in Rats with TK 10617. Ciba-Geigy Ltd. Toxicology, Project no. 821059, August 1983. [2]Wilson, J.G., in: Teratology, Principles and Techniques; J.G. Wilson and J. Warkany eds., The University of Chicago Press, Chicago, 1965, pp. 262-277. [3]Dawson, A.B., Stain Tech. 1 (1926), 123-124. [4]Salewski, E., Arch. Ex. Path. Pharmak. 247 (1964), pp. 367-368. [5]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. REPRODUCTIVE TOXICITY

The requirement for reproductive toxicity testing is met by the availability of 90-day repeat dose testing with appropriate analysis of reproductive organs and a developmental toxicity test. For this chemical, reproductive organs were analysed in a 90-day repeat dose study with rats. This summary describes the available repeat dose testing.

One repeat dose study has sufficient data for review (see section 16 for details of testing):

3-Month Toxicity Study in Rats. GU Project No. 821056, Ciba-Geigy Limited, Basel, Switzerland, January 20, 1984.

Reproductive organs were analyzed in the 90-day repeat dose study with rats cited above. Treatment-related adverse effects on reproductive organs were not observed in this study. The details of the evaluation of the reproductive organs from this study are summarized below.

3-Month Toxicity Study in Rats, 1984:

In this 3-month oral toxicity study in rats, all major organs were examined grossly and microscopically.The details of reproductive organs examined in this study are specified and these results are relevant to evaluation of potential reproductive effects. The results of the statistical analysis conducted on absolute gonad weights, organ to body weight, and organ to brain weight are summarized in the following tables. The mean body weight gain of all treated male and female groups was similar to that of the respective control groups, except a marginally lower weight increase in treated male group 4 (10,000 ppm), which however was not significant and within a range of 10% of the control value. This correlates with testes weight loss, where a slight decrease of testes weight was noted in treated male group 4 (10,000 ppm). This organ weight loss may have resulted from the general stress that reduced male body weights in this high dose group. There were no histopathological effects noted for the testes.

Overall, the test substance had no apparent effect on reproductive organs of either sex.

Organ Weight and Ratio data
Summary of Mean Values
Organ: Gonads (Male Rats)

Dietary Level (ppm)	Organ Weight (in G) NO	Mean	Organ / Body Weight Ratio (%) NO	Mean	Organ / Brain Weight Ratio (%) NO	Mean
Control	20	3.821	20	0.840	20	159.183
400	20	3.530	20	0.779	20	147.206
2000	19	3.616	19	0.809	19	148.293*
10,000	20	3.425*	20	0.792	20	140.611*

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUP (<0.050)

Organ Weight and Ratio data
Summary of Mean Values
Organ: Gonads (Female Rats)

Dietary Level (ppm)	Organ Weight (in G) NO	Mean	Organ / Body Weight Ratio (%) NO	Mean	Organ / Brain Weight Ratio (%) NO	Mean
Control	20	0.174	20	0.058	20	7.495
400	20	0.190	20	0.062	20	8.196
2000	20	0.175	20	0.056	20	7.419
10,000	20	0.177	20	0.060	20	7.662

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUP (<0.050)

Statistical Analysis of Mean Organ Weight and Ratios
Organ: Gonads (Male Rats)

Dietary Level (ppm)		Control	400	2000	10,000
Organ Weight (in G)	N MEAN SD MIN MAX REMARKS	20 3.821 0.318 3.105 4.335	20 3.530 0.580 1.393 4.376	19 3.616 0.273 3.219 4.267	20 3.425 0.576 1.321 4.164*
Organ / Body Weight Ratio (%)	N MEAN SD MIN MAX REMARKS	20 0.840 0.090 0.653 1.014	20 0.779 0.150 0.229 0.945	19 0.809 0.077 0.673 0.966	20 0.792 0.149 0.293 0.993
Organ / Brain Weight Ratio (%)	N MEAN SD MIN MAX REMARKS	20 159.183 12.701 134.590 184.704	20 147.206 27.430 54.799 200.336	19 148.293 13.983 125.037 175.799	20 140.611 24.414 54.073 172.341*

Statistical Analysis of Mean Organ Weight and Ratios
Organ: Gonads (Female Rats)

Dietary Level (ppm)		Control	400	2000	10,000
Organ Weight (in G)	N MEAN SD MIN MAX REMARKS	20 0.174 0.028 0.128 0.252	20 0.190 0.081 0.141 0.525	20 0.175 0.022 0.137 0.211	20 0.177 0.021 0.143 0.227
Organ / Body Weight Ratio (%)	N MEAN SD MIN MAX REMARKS	20 0.058 0.010 0.041 0.082	20 0.062 0.033 0.044 0.199	20 0.056 0.009 0.040 0.069	20 0.060 0.009 0.039 0.082
Organ / Brain Weight Ratio (%)	N MEAN SD MIN MAX REMARKS	20 7.495 1.179 5.499 10.198	20 8.196 3.450 6.044 22.465	20 7.419 0.800 5.737 8.676	20 7.662 1.111 5.729 9.874

The pathological and microscopic/ macroscopic examination was carried out on testes, prostate, uterus, fallopian tubes and ovaries along with other organs. No outstanding differences were noted between control and test groups. Macroscopic findings are summarized in the following table.

Summary of Macroscopic and Microscopic Findings

Organ: Gonads

Macroscopic and Microscopic Findings	Control		400 ppm		2000 ppm		10000 ppm	
	M	F	M	F	M	F	M	F
Reproductive System								
Testes								
Small, Bilateral	-	-	-	-	1	-	1	-
Spermatogenesis Reduced	-	-	1	-	1	-	1	-
Prostate								
Inflammatory Cell Infiltration	-	-	-	-	1	-	-	-
Ovaries								
Cyst, < 1 Cm	-	-	1	-	-	-	-	-
Corpus Luteum Cyst	-	-	-	-	-	1	-	-
Serous Cyst	-	-	-	1	-	-	-	-
Uterus								
Dilation	-	-	-	-	-	-	-	1
Fallopian Tube								
Inflammation with Fibrosis	-	-	-	-	-	1	-	-

Taken with the existing developmental toxicity study described in 17(A), the requirement for reproduction and developmental toxicity testing is fulfilled for CAS no. 32687-78-8.

GENERAL REFERENCE

Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

Definition of codes

1 = Valid without restriction
1a: GLP guideline study
1b: Comparable to guideline study
1c: Meets national standard methods (AFNOR/DIN)
1d: Meets generally accepted scientific standards and is described in sufficient detail

2 = Valid with restriction
2a: Guideline study without detailed documentation
2b: Guideline study with acceptable restrictions
2c: Comparable to guideline study with acceptable restrictions
2d: Meets national standard methods with acceptable restrictions
2e: Meets generally accepted scientific standards, well documented and acceptable for assessment
2f: Accepted calculation method
2g: Data from Handbook or collection of data

3 = Invalid
3a: Documentation insufficient for assessment
3b: Significant methodological deficiencies
3c: Unsuitable test system

4 = Not assignable
4a: Abstract
4b: Secondary literature
4c: Original reference not yet available
4d: Original reference in foreign language
4e: Documentation in sufficient for assessment